ELECTIVE (SSC5a) REPORT (1200 words)

A report that addresses the above four objectives should be written below. Your Elective supervisor will assess this.

Objective 1: Describe the prevalence and prognosis of harlequin ichthyosis in the UK and discuss this in the context of global health.

Harlequin ichthyosis (HI) is an autosomal-recessive skin disorder characterised by densely keratinised "armor-like" skin plates covering the body and separated by fissuring. The thickened skin is tight and constricts underlying tissue to precipitate contraction abnormalities of the eyes (eyelid eversion; ectropion), ears (flattening) and mouth (lip eversion; eclabion). The skin's discontinuity prevents it functioning as a physiological barrier against pathogens and fluid loss, rendering patients susceptible to infection and dehydration. HI is a result of mutation of the ATP adenosine triphosphate Binding Cassette A12 gene (ABCA12)[1], which encodes a protein responsible for transmembrane lipid transport as is consistent with HI's characteristic epidermal lipid localisation abnormalities. HI is rare with an incidence rate of 1 in 300,000 births and a 1.15:1 female: male ratio[2], though it occurs across all ethnic groups and increased local prevalences have been attributed to consanguinity.[3] Of the ~200 cases reported, 45 were included in a multinational retrospective study and the highest incidences were observed among British ethnicities (e.g. British Pakistani, 22.2%; British White, 11.1%; British Somali, 6.7%; British Bangladeshi, 4.4%[3]), though fewer than 5 cases per year occur in the UK.[2] While the condition has historically been reported to have a high neonatal mortality rate[4], Rajpopat et al.'s analysis found an overall survival rate of 56%[3] that may be attributed to recent advances in management discussed in Objective 2. The 44% of liveborn neonates who do not survive typically succumb to sepsis and/ or respiratory failure.[3] The condition's course among those surviving into infancy typically consists of hyperkeratotic plaque shedding, which is succeeded by erythroderma and generalised skin scaling, with associated alopecia alongside ectropion and eclabium persisting from the neonatal period. External auditory canal blockage, ophthalmological (conjunctivitis, strabismus, nystagmus) problems and developmental delay in speech, language and fine and gross motor skills can manifest as the child grows, alongside a susceptibility to skin infection and anaemia, and inflammatory arthritis and joint contractures can develop in teenage patients. The survivors reported in Rajpopat's analysis, comprising 56% of all cases, ranged from 10 months to 25 years in age[3] and the oldest living patient, Nusrit "Nelly" Shaheen, is currently 38 years old[5]; the lifespan of patients may be improving with optimised therapeutic intervention (discussed in Objective 2).

Objective 2: Outline the clinical management of and wider health provision for harlequin ichthyosis and how this compares globally.

Neonates with HI require immediate intensive care in an incubator at a temperature balancing heat loss with impaired sweating, at a humidity balancing transdermal evaporation with the risk of enabling skin infection, with supportive intravenous fluids and routine monitoring for electrolyte imbalance.[6] The skin is moisturised and cleaned via frequent bathing in water supplemented with emulsifying ointments and antiseptics, in addition to topical emollients routinely applied.[2] Breathing may be compromised through nasal obstruction or the pain of ventilating with truncal fissures, which may require intubation or analgesia respectively.[6] Further supportive treatments include nasogastric feeding to address jaw immobility[2] and prophylactic antibiotics to combat sepsis.[6]

As with other keratinisation disorders, systemic retinoids have emerged as a recent mainstay of therapeutic intervention.[7] Retinoids are vitamin A vitamers which regulate epithelial cell growth, and early systemic administration can accomplish accelerated skin plaque shedding and the associated benefits of reduced skin contraction in HI.[8] Despite their drawbacks, such as acitretin's teratogenicity and nervous, musculoskeletal and gastrointestinal toxicity[9, 10], their use may crucially promote survival, as Rajpopat et al. suggest in the above-discussed analysis; with early introduction, 83% of those given systemic retinoids survived while 76% of those not given retinoids succumbed to their HI.[3] Beyond the particularly challenging neonatal period, survivors have benefited from frequent emollient application, surgical intervention for contractions, close monitoring of nutrition and vitamin D and psychological support, and

therefore ultimately demonstrated the ability to lead normal, independent lives.[2]

Despite its clear importance in the management of HI, the availability of sufficient funding and personnel required for such neonatal intensive care cannot be assumed worldwide; neonatal mortality is higher in developing countries due to limited financial and human resources.[11] The healthcare systems of developing countries typically operate with fewer physicians per capita and a greater proportion of births occur at home without immediate access to neonatal intensive care.[12] Even should such care be accessible, it may not be affordable without local access to free healthcare.[13] Willingness to engage in healthcare may further be influenced by social stigma; some societies outcast those with skin conditions due to their perceived association with spiritual affliction[14], and medical professionals may even fear their patients.[15] It is clear that progress is being made in the management of HI and that this is reflected in its prognosis, but also that the availability of this care is not equal worldwide.

Objective 3: Discuss the various approaches to and global viability of prenatal diagnosis of harlequin ichthyosis.

Prior to identification of ABCA12 mutation as a determinant of HI, formal prenatal diagnosis required fetal skin biopsy and electron microscopy.[16] However, recognising the genetic basis for HI has allowed application of DNA-based approaches to prenatal diagnosis regularly employed with other pathologies (e.g. Down's Syndrome) such as chorionic villus or amniotic fluid cell sampling.[6] These techniques confer a reduced risk to both the parent and fetus, and can be performed earlier in the pregnancy.[17] As heterozygous carriers are asymptomatic, requests for prenatal diagnosis are typically requested by parents who have previously had a child with HI and are determined to both carry causative mutations. While chorionic villus samples are a viable source of fetal DNA, amniotic fluid samples have been used in every case of prenatal genetic HI diagnosis to date.[17] With the identification of ABCA12 as an HI determinant, preimplantation genetic diagnosis is now possible for those using in vitro fertilisation.[2, 18] In the past decade, sonographic imaging has become a viable approach. While not diagnostic, conventional 2D sonography typically employed in routine prenatal scans can detect anomalies to indicate diagnostic use of 3D and 4D ultrasound, which can demonstrate features including abnormal ear, nose face morphology, a large open mouth, thickened skin and reduced fetal movement with joints apparently restricted in a semi-flexed position.[19] Prenatal diagnosis has exhibited stepwise technology-dependent progression in developed countries, but the required technology is not available for this application in developing countries and the problem is compounded by both reduced maternal engagement in even routine prenatal care and, even where maximal engagement occurs, low quality content of care.[20] There is inequality in the viability of prenatal diagnosis of HI globally, and addressing this would require not only increased access to high end analytical technology in less economically developed regions but further systemic developments in the education surrounding and delivery of prenatal healthcare.

Objective 4: Develop my repertoire in wet lab research through the use of new technology and techniques. Specifically: Generate three-dimensional organotypic cultures of HI epidermis as per the group's established protocol using their in-house CRISPRCAS9 ABCA12 KO cell line, and treat these with individual cytokines (or diluent for control). Following cytokine treatment, explore IL36 signaling with immunostaining and the model's epidermal structural integrity with various histological stains and Lucifer Yellow Dye exclusion test.

While the techniques I developed my skills in shifted from the above according to the immediate availability of equipment and personnel, my repertoire was still significantly enhanced as is outlined in the below overview of my activities on placement. The first 2 weeks were dedicated to exploring RNAseq data comparing HI and normal skin, for which I learned to use a new platform, StringDB[21], to explore functional partnerships between the proteins of differentially expressed genes in the data. Given the limited duration of this component of the project, I set an arbitrary threshold of 5-fold change in differential expression to select for the most significantly altered genes and, among them, attempted to identify those most associated with ABCA12.

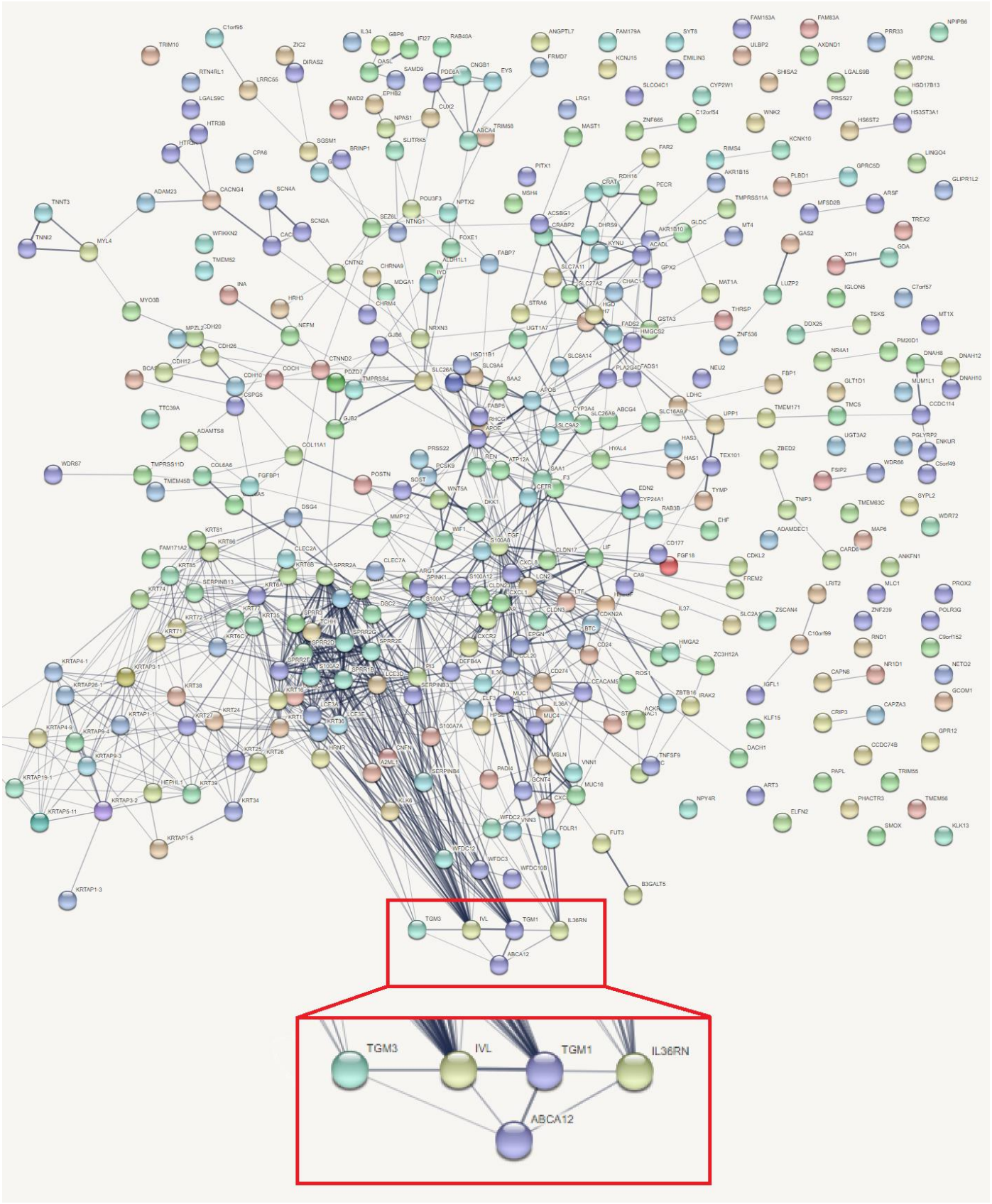
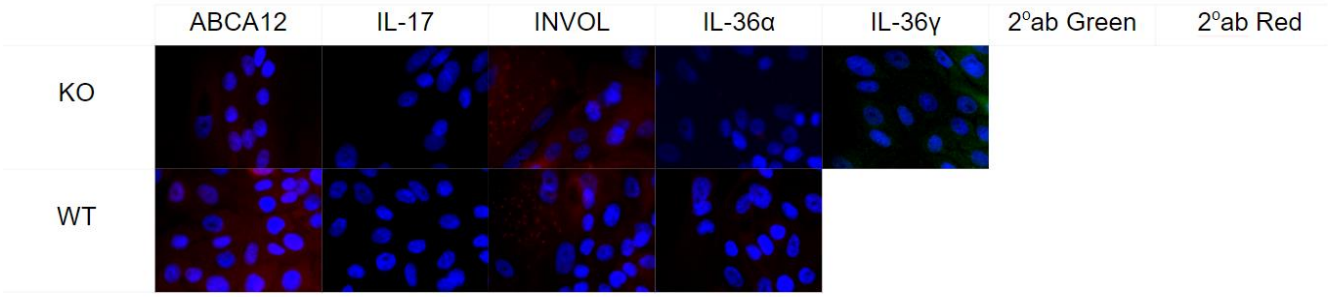


Figure 1 StringDB functional network analysis identifies ABCA12-associated genes among those differentially expressed between HI and normal skin. IL36RN, TGM1, TGM3 and IVL emerged as the primary functional associates of ABCA12 among the differentially expressed genes, and themselves shared various functional associations between each other and many other genes differentially expressed between HI and normal skin.

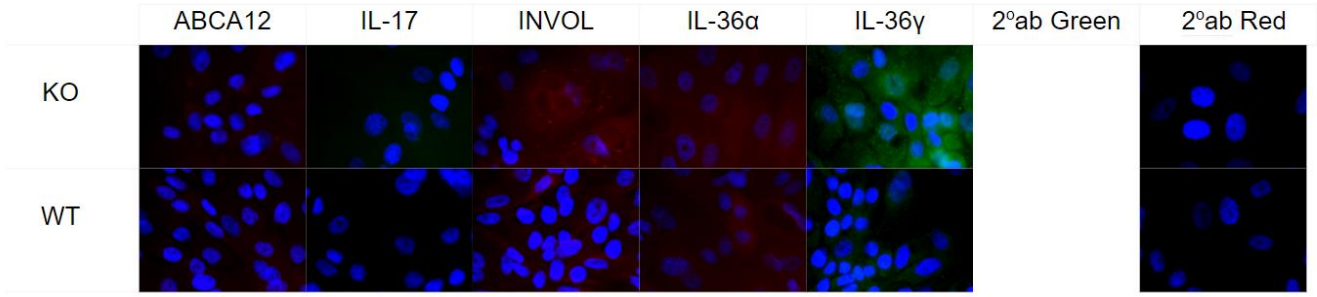
Figure 2 IL-36 and IFN-γ increase the abundance of IL-36γ in an ABCA12 knockout HI model *in vitro*. Immunofluorescence of various proteins of interest (ABCA12, IL-17A, Involucrin, IL-36α and IL-36γ) in IFN-γ-, IL-17A- or IL-36-treated immortalised human keratinocytes N/TERT cell lines with and without CRISPr/CAS9-mediated ABCA12 knockout, modeling HI and normal skin respectively, revealed an apparent increase in IL-36 abundance in response to 24 hours of exposure to either IFN-γ or IL-36 specifically in the ABCA12 knockout. Dated by day of image acquisition.

11/5/22

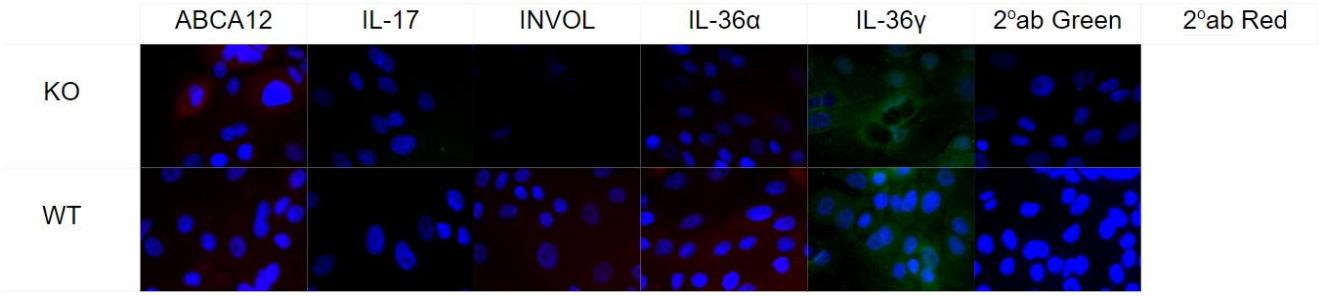
Control (Unstimulated)



IFNγ Stimulated

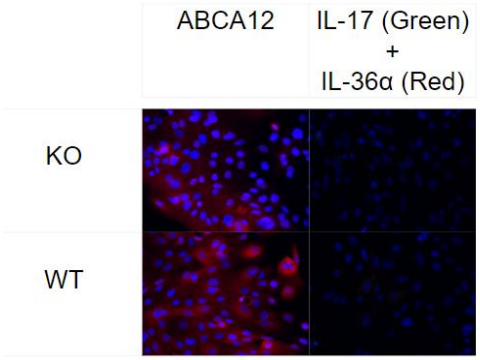


IL-17 Stimulated

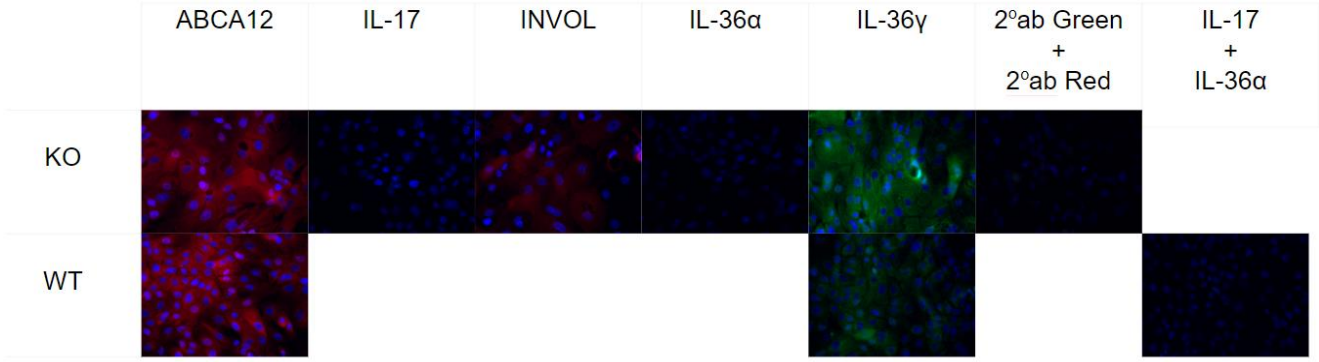


13/5/22

Control (Unstimulated)



IL-36 Stimulated



Four genes emerged as the primary functional associates of ABCA12 among the genes differentially expressed between HI and normal skin; IL36RN, TGM1, TGM 3 and IVL. Given my research objective, to stimulate an HI model with various cytokines and examine the effects on IL36 signaling and the skin barrier, IL36RN was the most interesting. Its functional association with ABCA12 as identified by StringDB is based primarily on their co-expression (co-expression score 0.066[21]). It encodes interleukin 36 receptor antagonist (IL-36Ra), which endogenously inhibits pro-inflammatory IL-36α, IL-36β and IL-36γ to regulate inflammation.[22] Should disrupted ABCA12 expression be coupled with disrupted IL36RN expression, dysregulation of IL-36 signaling may contribute to HI pathology. While TGM1, TGM3 and IVL have known roles in ichthyoses and dysfunctional cornification[23] and the relevance of this to HI is important, it is beyond the scope of this report.

Upon placement in the lab, my work began with maintaining the health of HI model and control cell lines (immortalised human keratinocytes N/TERT cell lines with and without CRISPr/CAS9-mediated ABCA12 knockout respectively), requiring routine media changes and passaging. Experiments required counting cells and plating them to coverslips (for subsequent immunostaining) in controlled amounts, then exposing them to IL36 (20 ng/ml), IL17A (100 ng/ml) and IFN-γ (2.5 ng/ml) for 24 hours at 37°C using stock solutions I prepared. Following stimulation, I fixed the cells and applied primary and secondary antibodies over 2 days for immunostaining to explore their responses, which were visualized on a Leica DM 5000-D microscope and captured with MetaMorph software (Fig. 2). Findings of note included the apparent increase in abundance of IL-36γ in response to 24 hours of IFNγ or IL-36 treatment occurring specifically in the ABCA12 knockout model of HI. It is also notable that ABCA12 appears to be expressed equally in both the ABCA12 knockout and wild-type cell lines, and that the quality of my image acquisition improved significantly after a day's experience, as is evident in the higher quality of the later images captured on my second day at the microscope.

The word limit for Objective 4 is 300, yet each of the many procedures described above would require more than this and further techniques (e.g. Western Blot) in which I developed my technique have gone unmentioned. The same is true of the results, which have prompted the use of the identified stimulating cytokines in organotypic cultures, although this data will not make it into this report. While it cannot be fully expressed presently, the wet lab experience gained here at Blizard has been comprehensive and extremely valuable.

References

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